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(21) International Application Number: PCT/US98/22679 (22) International Filing Date: 26 October 1998 (26.10.98) (30) Priority Data: 60/063,646 27 October 1997 (27.10.97) US (71) Applicant (for all designated States except US): ELI LILLY AND COMPANY [US/US]; Lilly Corporate Center, Indianapolis, IN 46285 (US). (72) Inventors; and (75) Inventors/Applicants (for US only): DENNEY, Michael, Lyle [US/US]; 6867 East Urmeyville Road, Franklin, IN 46131 (US). MORIN, John, Michael, Jr. [US/US]; 9 Roselawn Avenue, Brownsburg, IN 46112 (US). SALL, Daniel, Jon [US/US]; 376 Leisure Lane, Greenwood, IN 46142 (US). SAWYER, Jason, Scott [US/US]; 5718 North Winthrop Avenue, Indianapolis, IN 46220 (US). (74) Agents: BENJAMIN, Roger, S. et al.; Eli Lilly and Company, Lilly Corporate Center, Indianapolis, IN 46285 (US).		(81) Designated States: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG). Published <i>With international search report.</i>
(54) Title: MORPHOLINO-N-ETHYL ESTER PRODRUGS OF INDOLE sPLA ₂ INHIBITORS (57) Abstract The compound, ((3-(2-amino-1,2-dioxoethyl)-1-((1,1'-biphenyl)-3-ylmethyl)-2-methyl-1H-indol-4-yl)oxy)acetic acid morpholino-N-ethyl ester, is disclosed together with its use as a highly bioavailable indole compound for inhibiting sPLA ₂ mediated release of fatty acids for treatment of conditions such as septic shock.		

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MORPHOLINO-N-ETHYL ESTER PRODRUGS OF
INDOLE sPLA₂ INHIBITORS

FIELD OF THE INVENTION

- 5 This invention relates to a novel prodrug form of sPLA₂ inhibitor having exceptionally high bioavailability.

BACKGROUND OF THE INVENTION

- 10 The structure and physical properties of human non-pancreatic secretory phospholipase A₂ (hereinafter called, "sPLA₂") has been thoroughly described in two articles, namely, "Cloning and Recombinant Expression of Phospholipase A₂ Present in Rheumatoid Arthritic Synovial Fluid" by Seilhamer, Jeffrey J.; Pruzanski, Waldemar; Vadas Peter; 15 Plant, Shelley; Miller, Judy A.; Kloss, Jean; and Johnson, Lorin K.; *The Journal of Biological Chemistry*, Vol. 264, No. 10, Issue of April 5, pp. 5335-5338, 1989; and "Structure and Properties of a Human Non-pancreatic Phospholipase A₂" by Kramer, Ruth M.; Hession, Catherine; 20 Johansen, Berit; Hayes, Gretchen; McGray, Paula; Chow, E. Pingchang; Tizard, Richard; and Pepinsky, R. Blake; *The Journal of Biological Chemistry*, Vol. 264, No. 10, Issue of April 5, pp. 5768-5775, 1989; the disclosures of which are incorporated herein by reference.

- 25 It is believed that sPLA₂ is a rate limiting enzyme in the arachidonic acid cascade which hydrolyzes membrane phospholipids. Thus, it is important to develop compounds

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which inhibit sPLA₂ mediated release of fatty acids (e.g., arachidonic acid) and are highly bioavailable in mammals, especially humans. Such compounds are of value in general treatment of conditions induced and/or maintained by
5 overproduction of sPLA₂; such as septic shock, adult respiratory distress syndrome, pancreatitis, trauma, bronchial asthma, allergic rhinitis, rheumatoid arthritis, etc.

Therapeutic agents that may be given orally are, in
10 general, greatly preferred and have enhanced commercial potential because of their inherent ease of use.

Prodrugs are forms of therapeutic agents sometimes used to improve performance of known therapeutic agents. For example, mycophenolic acid is reported to be improved by
15 conversion to its morpholino ethyl type prodrug (see, article, "Mycophenolate mofetil" by James J. Lipsky, *The Lancet*, Vol 348, November 16, 1997, pg. 1357-1359).

U.S. Patent No. 5,654,326 describes certain indole type sPLA₂ inhibitors. In particular, this patent exemplifies
20 the methyl ester of, ((3-(2-amino-1,2-dioxoethyl)-1-((1,1'-biphenyl)-3-ylmethyl)-2-methyl-1H-indol-4-yl)oxy)acetic acid.

It is desirable to develop more highly available sPLA₂ inhibitors, particularly those suitable for oral
25 administration.

SUMMARY OF THE INVENTION

This invention is the novel compound, ((3-(2-amino-1,2-dioxoethyl)-1-((1,1'-biphenyl)-3-ylmethyl)-2-methyl-1H-
30 indol-4-yl)oxy)acetic acid morpholino-N-ethyl ester; which is highly bioavailable by oral administration.

This invention is also a pharmaceutical formulation containing the novel compound of the invention.

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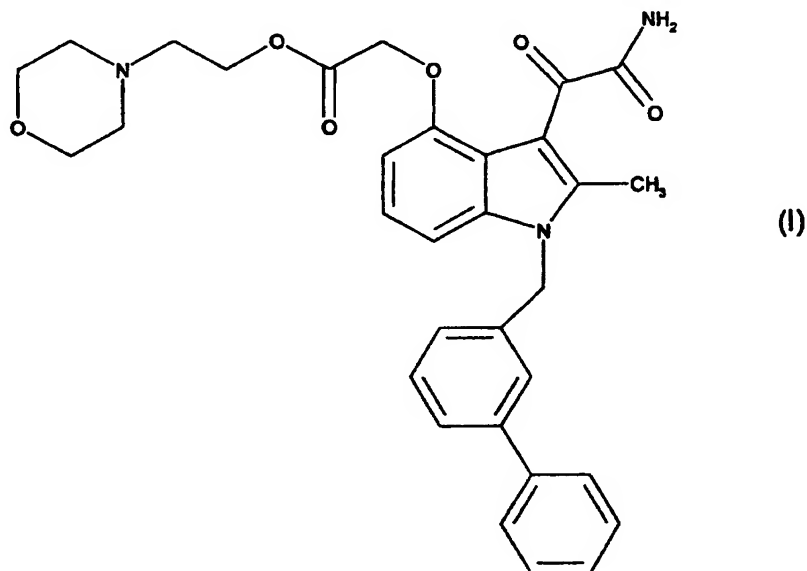
This invention is also a method of inhibiting sPLA₂ mediated release of fatty acid by contacting sPLA₂ with the novel compound of the invention.

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DETAILED DESCRIPTION OF THE INVENTION

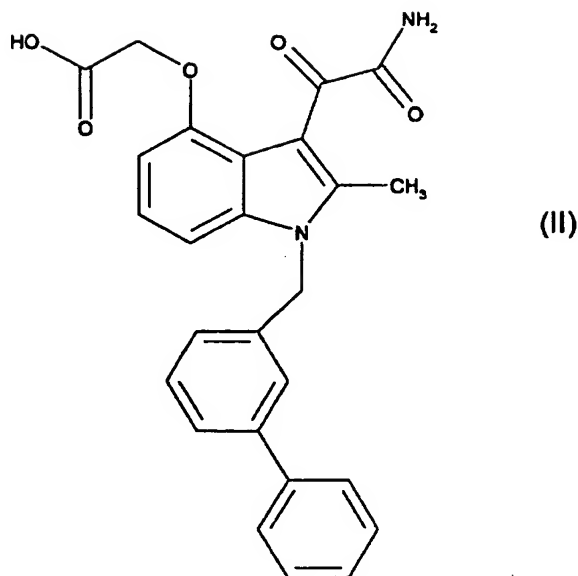
THE 1H-INDOLE-3-GLYOXYLAMIDE COMPOUND OF THE INVENTION:

The compound of the invention ((3-(2-amino-1,2-dioxoethyl)-1-((1,1'-biphenyl)-3-ylmethyl)-2-methyl-1H-indol-4-yl)oxy)acetic acid morpholino-N-ethyl ester;
10 is represented by the structural formula (I);



The morpholino-N-ethyl ester (I) is an ester form of
15 known sPLA₂ inhibitor ((3-(2-amino-1,2-dioxoethyl)-1-((1,1'-biphenyl)-3-ylmethyl)-2-methyl-1H-indol-4-yl)oxy)acetic acid, represented by the structural formula (II), below;

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The compound of formula (II) is described in Example 4 of U.S. Patent No. 5,654,326 (the disclosure of which is incorporated herein by reference) and European Patent Application No. 95302166.4, Publication No. 0 675 110 (publ., 4 October 1995).

Prodrugs are derivatives of therapeutic agents which have chemically or metabolically cleavable groups and become under physiological conditions known compounds of therapeutic effectiveness.

It is a discovery of this invention that the compound of formula (I) is highly bioavailable upon oral administration compared to other common ester type prodrugs.

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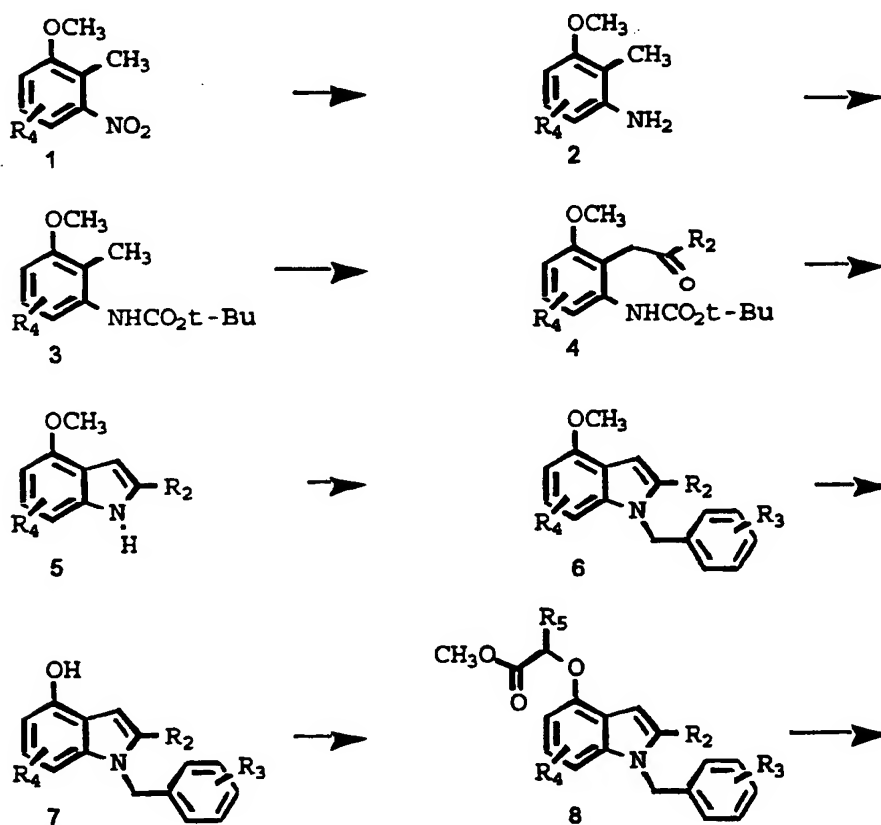
SYNTHESIS OF THE COMPOUND OF THE INVENTION:

The synthesis of ((3-(2-amino-1,2-dioxoethyl)-1-((1,1'-biphenyl)-3-ylmethyl)-2-methyl-1H-indol-4-yl)oxy)acetic acid, morpholino-N-ethyl ester (compound of formula I, supra.) uses as starting material ((3-(2-amino-1,2-dioxoethyl)-1-((1,1'-biphenyl)-3-ylmethyl)-2-methyl-1H-indol-4-yl)oxy)acetic acid, or a salt thereof (compound of

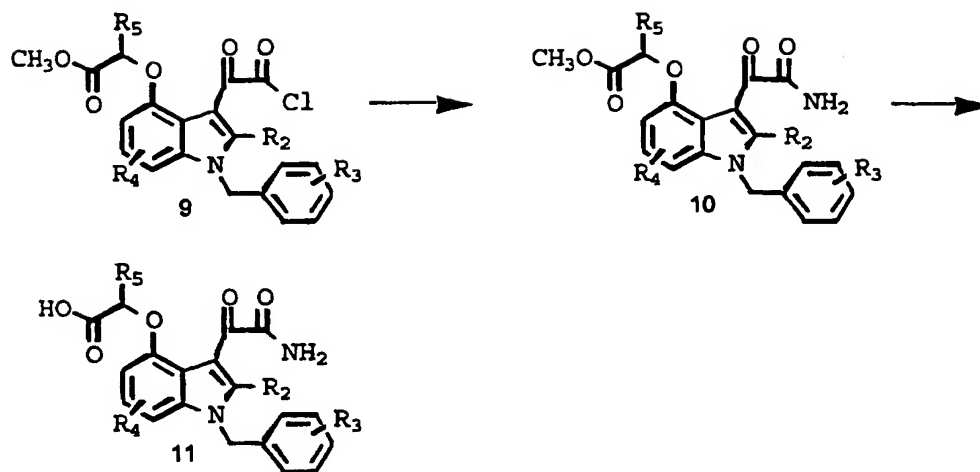
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formula II, supra.). This starting material may be prepared by the reaction schemes or method of Example 4 of U.S. Patent No. 5,654,326 (the disclosure of which is incorporated herein by reference). Similar methods are shown in European Patent Application No. 95302166.4, Publication No. 0 675 110 (publ., 4 October 1995). Other methods well known and recorded in the chemical literature may also be used for preparing the starting material. Procedures useful for the synthesis of the starting material are shown in both Scheme 1 and Example 1 set out below:

Scheme 1



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To obtain the glyoxylamides substituted in the 4-position with an acidic function linked through an oxygen atom, the reactions outlined in scheme 1 are used (for conversions 1 through 5, see ref. Robin D. Clark, Joseph M. Muchowski, Lawrence E. Fisher, Lee A. Flippin, David B. Repke, Michel Souchet, *Synthesis*, 1991, 871-878, the disclosures of which are incorporated herein by reference).

The ortho-nitrotoluene, 1, is readily reduced to the 2-methylaniline, 2, using palladium on carbon as catalyst. The reduction can be carried out in ethanol or tetrahydrofuran (THF) or a combination of both, using a low pressure of hydrogen. The aniline, 2, on heating with di-tert-butyl dicarbonate in THF at reflux temperature is converted to the N-tert-butyloxycarbonyl derivative, 3, in good yield. The dilithium salt of the dianion of 3 is generated at -40 to -20°C in THF using sec-butyllithium and reacted with the appropriately substituted N-methoxy-N-methylalkanamide. This product, 4, may be purified by crystallization from hexane, or reacted directly with trifluoroacetic acid in methylene chloride to give the 1,3-unsubstituted indole 5. The 1,3-unsubstituted indole 5 is reacted with sodium hydride in dimethylformamide at room

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temperature (20-25°C) for 0.5-1.0 hour. The resulting sodium salt of 5 is treated with an equivalent of arylmethyl halide and the mixture stirred at a temperature range of 0-100°C, usually at ambient room temperature, for a period of 4 to 36 hours to give the 1-arylmethylindole, 6. This indole, 6, is O-demethylated by stirring with boron tribromide in methylene chloride for approximately 5 hours (see ref. Tsung-Ying Shem and Charles A Winter, *Adv. Drug Res.*, 1977, 12, 176, the disclosure of which is incorporated herein by reference). The 4-hydroxyindole, 7, is alkylated with an alpha bromoalkanoic acid ester in dimethylformamide (DMF) using sodium hydride as a base, with reactions conditions similar to that described for the conversion of 5 to 6. The α -((indol-4-yl)oxy)alkanoic acid ester, 8, is reacted with oxalyl chloride in methylene chloride to give 9, which is not purified but reacted directly with ammonia to give the glyoxamide 10. This product is hydrolyzed using 1N sodium hydroxide in methanol. The final glyoxylamide, 11, is isolated either as the free carboxylic acid or as its sodium salt or in both forms.

Example 1

Method of Preparing ((3-(2-amino-1,2-dioxoethyl)-1-((1,1'-biphenyl)-3-ylmethyl)-2-methyl-1H-indol-4-yl)oxy)acetic acid.

Part A. Preparation of 1-((1,1'-biphenyl)-3-ylmethyl)-2-methyl-4-methoxy-1H-indole.

805 mg (5 mmol) of 4-methoxy-2-methyl-1H-indole is reacted with 200mg (5 mmol) of 60% NaH/mineral oil (washing with hexane before adding DMF) in 15 mL of DMF and after stirring for 0.5 hour, 1.0 g (5 mmol) of 3-(chloromethyl)biphenyl is added. The mixture is stirred at room temperature for 18 hours, diluted with water and

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extracted with ethyl acetate. The ethyl acetate solution is washed with brine, dried (MgSO_4) and after concentrating at reduced pressure, the residue is chromatographed on silica gel eluting with 20% EtOAc/hexane to give 1.25g

5 (76% yield) of

1-((1,1'-biphenyl)-3-ylmethyl)-2-methyl-4-methoxy-1H-indole
mp, 127°-131°C.

Part B. Preparation of 1-((1,1'-Biphenyl)-3-ylmethyl)-2-methyl-4-hydroxy-1H-indole.

10 A solution of 125 mg (3.8 mmol) of 1-((1,1'-biphenyl)-3-ylmethyl)-2-methyl-4-methoxy-1H-indole is O-demethylated by treating it with 15 mL of 1M $\text{BBr}_3/\text{CH}_2\text{Cl}_2$. The reaction mixture is stirred at room temperature for 5 hours and
15 concentrated at reduced pressure. The crude product is chromatographed on silica gel and is eluted with 20% EtOAc/hexane to give 1030 mg (87% yield) of 1-((1,1'-biphenyl)-3-ylmethyl)-2-methyl-4-hydroxy-1H-indole.

20 Part C. Preparation of ((1-((1,1'-Biphenyl)-3-ylmethyl)-2-methyl-1H-indol-4-yl)oxy)acetic acid methyl ester.

1-((1,1'-Biphenyl)-3-ylmethyl)-4-hydroxy-2-methyl-1H-indole (1030 mg, 3.3 mmol) is alkylated by treating with
25 0.31 mL (3.3 mmol) of methyl bromoacetate and 132mg (3.3 mmol) and 132mg (3.3 mmol) of 60% NaH/mineral oil in DMF and stirring maintained for about 17 hours. The mixture is diluted with water and extracted with ethyl acetate. The ethyl acetate solution is washed with brine, dried (MgSO_4),
30 and concentrated at reduced pressure. The product is purified by chromatography over silica gel eluting with 20% EtOAc/hexane, to give 1000 mg (79% yield) of ((1-((1,1'-biphenyl)-3-ylmethyl)-2-methyl-1H-indol-4-yl)oxy)acetic acid methyl ester; mp 99°-102°C.

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Part D. Preparation of ((3-(2-Amino-1,2-dioxoethyl)-1-((1,1'-biphenyl)-3-ylmethyl)-2-methyl-1H-indol-4-yl)oxy)acetic acid methyl ester.

5 Oxalyl chloride (0.23 mL, 2.6 mmol) is added to 1000mg (2.6 mmol) of ((1-((1,1'-biphenyl)-3-ylmethyl)-2-methyl-1H-indol-4-yl)oxy)acetic acid methyl ester in 15 mL of methylene chloride and the mixture is stirred for 1.3 hours at room temperature. The mixture is concentrated at reduced
10 pressure, the residue redissolved in 15 mL of methylene chloride, ammonia bubbled in for 0.25 hours, stirred for 0.25 hours and concentrated. The residue is stirred with EtOAc/water and the undissolved material filtered to give
15 300mg of ((3-(2-amino-1,2-dioxoethyl)-1-((1,1'-biphenyl)-3-ylmethyl)-2-methyl-1H-indol-4-yl)oxy)acetic acid methyl ester. The residue was chromatographed on silica gel eluting with EtOAc to give an additional 671 mg of product; mp, 175°-179°C. The total combined yield of product was 82%.

20 Part E. Preparation of ((3-(2-amino-1,2-dioxoethyl)-1-((1,1'-biphenyl)-3-ylmethyl)-2-methyl-1H-indol-4-yl)oxy)acetic acid.

 A mixture of 956mg (2.1 mmol) of ((3-(2-amino-1,2-dioxoethyl)-1-((1,1'-biphenyl)-3-ylmethyl)-2-methyl-1H-
25 indol-4-yl)oxy)acetic acid methyl ester is hydrolyzed at reflux in 10 mL of 1N NaOH and 20 mL of MeOH to give 403 mg (41% yield) of ((3-(2-amino-1,2-dioxoethyl)-1-((1,1'-biphenyl)-3-ylmethyl)-2-methyl-1H-indol-4-yl)oxy)acetic acid, sodium salt, mp, greater than 265°C.

30 Analyses for C₂₆H₂₁N₂O₅Na:

 Calculated: C, 67.24; H, 4.56; N, 6.03

 Found: C, 67.20; H, 4.58; N, 6.03.

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There is also obtained 346 mg (37% yield) of ((3-(2-amino-1,2-dioxoethyl)-1-((1,1'-biphenyl)-3-ylmethyl)-2-methyl-1H-indol-4-yl)oxy)acetic acid, mp, 236°-238°C.

Analyses for C₂₆H₂₂N₂O₅:

5 Calculated: C, 70.58; H, 5.01; N, 6.33
 Found: C, 70.58; H, 5.25; N, 6.11.

Beginning with the indole starting material of formula (II) prepared by the above methods the ((3-(2-amino-1,2-dioxoethyl)-1-((1,1'-biphenyl)-3-ylmethyl)-2-methyl-1H-indol-4-yl)oxy)acetic acid, morpholino-N-ethyl ester compound of the invention is prepared by esterification of the acid or salt form of the starting material. Any ester forming method which is conventional in the chemical arts may be used. A suitable procedure used to prepare the compound of the invention is as follows:

Example 2

Preparation of ((3-(2-Amino-1,2-dioxoethyl)-1-((1,1'-biphenyl)-3-ylmethyl)-2-methyl-1H-indol-4-yl)oxy)acetic acid, morpholinylethyl ester.

In a flask containing 10 ml of dimethylformamide was added with stirring 133 mg. of 4-(2-chloroethyl)morpholine hydrochloride (available from Aldrich Chemical Co., Milwaukee, Wisconsin USA, Item No. C4,220-3) and 231 mg. of CsCO₃ and 300 mg. of ((3-(2-amino-1,2-dioxoethyl)-1-((1,1'-biphenyl)-3-ylmethyl)-2-methyl-1H-indol-4-yl)oxy)acetic acid, sodium salt. The slurry was heated to 60°C until a solution formed. Heating was continued overnight until reaction was complete. 20 ml of H₂O was added to the flask and the organic soluble phase extracted with three 20 ml. portions of ethyl acetate. The ethyl acetate solution was washed with water and dried over and dried over Na₂SO₄.

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Removal of solvent gave product verified by IR and NMR to be the title compound.

Molecular Formula: $C_{32}H_{22}N_3O_6$

Calculated % C = 69.17 H = 5.99 N = 7.56

5 Found % C = 69.23 H = 5.84 N = 7.27

FORMULATIONS SUITABLE FOR USE IN THE METHOD OF THE INVENTION

The $sPLA_2$ inhibitor of formula (I) used in the method of the invention is administered so as to make contact with
10 $sPLA_2$ in the body of the mammal being treated.

The preferred route of administration for the compound of this invention is orally, either as neat compound or as the active compound in a pharmaceutical formulation.

The $sPLA_2$ inhibitor can be administered alone, but is
15 generally administered with a pharmaceutical carrier or diluent selected on the basis of the chosen route of administration and standard pharmaceutical practice.

By "pharmaceutically acceptable" it is meant the carrier, diluent or excipient must be compatible with the
20 formula (II) $sPLA_2$ inhibitor ("active compound") in the formulation and not deleterious to the subject being treated.

For the pharmaceutical formulations any suitable carrier known in the art can be used. In such a
25 formulation, the carrier may be a solid, liquid, or mixture of a solid and a liquid. A solid carrier can be one or more substances which may also act as flavoring agents, lubricants, solubilizers, suspending agents, binders, tablet disintegrating agents and encapsulating material.

30 Tablets for oral administration may contain suitable excipients such as calcium carbonate, sodium carbonate, lactose, calcium phosphate, together with disintegrating agents, such as maize, starch, or alginic acid, and/or binding agents, for example, gelatin or acacia, and

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lubricating agents such as magnesium stearate, stearic acid, or talc. In tablets the sPLA₂ inhibitor is mixed with a carrier having the necessary binding properties in suitable proportions and compacted in the shape and size desired.

5 Sterile liquid form formulations include suspensions, emulsions, syrups and elixirs. The active compound can be dissolved or suspended in a pharmaceutically acceptable carrier, such as sterile water, saline, dextrose solution, sterile organic solvent or a mixture of both.

10 The active compound can be administered orally in solid dosage forms, such as capsules, tablets, and powders, or in liquid dosage forms, such as elixirs, syrups, and suspensions. It can also be administered parenterally, in sterile liquid dosage forms. It can also be administered by
15 inhalation in the form of a nasal spray or lung inhaler. It can also be administered topically as an ointment, cream, gel, paste, lotion, solution, spray, aerosol, liposome, or patch. Dosage forms used to administer the active compound usually contain suitable carriers, diluents, preservatives,
20 or other excipients, as described in Remington's Pharmaceutical Sciences, Mack Publishing Company, a standard reference text in the field.

Gelatin capsules may be prepared containing the active compound and powdered carriers, such as lactose, sucrose,
25 mannitol, starch, cellulose derivatives, magnesium stearate, stearic acid, and the like. Similar diluents can be used to make compressed tablets and powders. Both tablets and capsules can be manufactured as sustained release products to provide for continuous release of medication over a
30 period of hours. Compressed tablets can be sugar coated or film coated to mask any unpleasant taste and protect the tablet from the atmosphere, or enteric coated for selective disintegration in the gastrointestinal tract.

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For parenteral solutions, water, a suitable oil, saline, aqueous dextrose (glucose), and related sugar solutions and glycols such as propylene glycol or polyethylene glycols are suitable carriers for parenteral solutions. Solutions for parenteral administration contain the active compound, suitable stabilizing agents, and if necessary, buffer substances. Anti-oxidizing agents such as sodium bisulfite, sodium sulfite, or ascorbic acid either alone or combined are suitable stabilizing agents. Also used are citric acid and its salts and sodium EDTA. In addition, parenteral solutions can contain preservatives, such as benzalkonium chloride, methyl- or propyl-paraben, and chlorobutanol.

Formulations within the scope of this invention include the admixture of sPLA₂ inhibitor with a therapeutically effective amount of any therapeutically effective co-agents, such as other sPLA₂ inhibitors, leukotriene antagonists or cyclooxygenase inhibitors for treating the disease target.

PROPORTION AND WEIGHT OF ACTIVE COMPOUNDS USED IN THE METHOD OF THE INVENTION

The compound of formula (I) may be used at a concentration of 0.1 to 99.9 weight percent of the pharmaceutical formulation.

Preferably the pharmaceutical formulation is in unit dosage form. The unit dosage form can be a capsule or tablet itself, or the appropriate number of any of these. The quantity of active compound in a unit dose of composition may be varied or adjusted from about 0.1 to about 1000 milligrams or more according to the particular treatment involved.

Compositions (dosage forms) suitable for internal administration contain from about 1 milligram to about 500 milligrams of active compound per unit.

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Examples of useful pharmaceutical compositions and their proportions of ingredients are illustrated as follows:

Capsules: Capsules may be prepared by filling standard two-piece hard gelatin capsules each with 50 mg of powdered
5 active compound, 175 mg of lactose, 24 mg of talc, and 6 mg of magnesium stearate.

Soft Gelatin Capsules: A mixture of active compound in soybean oil is prepared and injected by means of a positive displacement pump into gelatin to form soft gelatin capsules
10 containing 50 mg of the active compound. The capsules are washed in petroleum ether and dried.

Tablets: Tablets may be prepared by conventional procedures so that the dosage unit is 50 mg of active compound, 6 mg of magnesium stearate, 70 mg of
15 microcrystalline cellulose, 11 mg of cornstarch, and 225 mg of lactose. Appropriate coatings may be applied to increase palatability or delay absorption. Tablets for oral administration may contain suitable excipients such as calcium carbonate, sodium carbonate, lactose, calcium
20 phosphate, together with disintegrating agents, such as maize, starch, or alginic acid, and/or binding agents, for example, gelatin or acacia, and lubricating agents such as magnesium stearate, stearic acid, or talc. The tablets preferably contain from about 1 to about 99 weight percent
25 of the active ingredient which is the novel compound of this invention. Suitable solid carriers are magnesium carbonate, magnesium stearate, talc, sugar lactose, pectin, dextrin, starch, gelatin, tragacanth, methyl cellulose, sodium carboxymethyl cellulose, low melting waxes, and cocoa
30 butter.

Suspensions: An aqueous suspension is prepared for oral administration so that each 5 ml contain 25 mg of finely divided active compound, 200 mg of sodium

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carboxymethyl cellulose, 5 mg of sodium benzoate, 1.0 g of sorbitol solution, U.S.P., and 0.025 mg of vanillin.

Injectables: A parenteral composition suitable for administration by injection is prepared by stirring 1.5% by weight of active compound in 10% by volume propylene glycol and water. The solution is sterilized by commonly used techniques.

Nasal Spray: An aqueous solution is prepared such that each 1 ml contains 10 mg of active compound, 1.8 mg methylparaben, 0.2 mg propylparaben and 10 mg methylcellulose. The solution is dispensed into 1 ml vials. The active compound may be used at a concentration of 0.1 to 99.9 weight percent of the formulation.

Aerosol formulations are capable of dispersing into particle sizes of from about 0.5 to about 10 microns and have sufficient sPLA₂ inhibitor to achieve concentrations of the inhibitor on the airway surfaces of from about 10^{-10} to 10^{-2} moles per liter.

The dosage administered will, of course, vary depending upon known factors such as the pharmacodynamic characteristics of the particular agent, and its mode and route of administration; age, health, and weight of the recipient; nature and extent of symptoms, kind of concurrent treatment, frequency of treatment, and the effect desired. Usually a daily dosage of active compound can be about 0.1 to 200 milligrams per kilogram of body weight. Ordinarily 0.5 to 50, and preferably 1 to 25 milligrams per kilogram per day given in divided doses 1 to 6 times a day or in sustained release form is effective to obtain desired results.

In general, the sPLA₂ inhibitor will be administered to a human so that a therapeutically effective amount is received. A therapeutically effective amount may coventionally be determined for an individual patient by

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administering the active compound in increasing doses and observing the effect on the patient.

Generally, the compound must be administered in a manner and a dose to achieve in the human a blood level
5 concentration of sPLA₂ inhibitor of from 10 to 3000 nanograms/ml, and preferably a concentration of 100 to 800 nanograms/ml.

The treatment regimen may be from a single dose to multiple doses taken for years. Oral dosing is preferred
10 for patient convenience and tolerance. With oral dosing, one to four oral doses per day, each from about 0.01 to 25 mg/kg of body weight with preferred doses being from about 0.1 mg/kg to about 2 mg/kg.

Parenteral administration (particularly, intravenous
15 administration) is often preferred in instances where rapid alleviation of patient distress is required. With parenteral administration doses of 0.01 to 100 mg/kg/day administered continuously or intermittently throughout the day may be used. For parenteral administration, the compound
20 may be administered in a physiologic saline vehicle (e.g., 0.9% normal saline, 0.45% normal saline, etc.) a dextrose vehicle (e.g., 5% dextrose in water), or a combination of saline and dextrose vehicle (0.9% normal saline in 5% dextrose).

25

USE AND PROPORTION OF ACTIVE COMPOUND IN THE METHOD OF THE INVENTION:

Another aspect of this invention is a method for treating septic shock, adult respiratory distress syndrome,
30 panceatitis, trauma, bronchial asthma, allergic rhinitis, rheumatoid arthritis, stroke, acute bronchitis, chronic bronchitis, acute bronchiolitis, chronic bronchiolitis, isteoarthritis, gout, spondylarthropathris, ankylosing spondylitis, Reiter's syndrome, psoriatic arthropathy,

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enteropathic spondylitis, juvenile arthropathy, juvenile ankylosing spondylitis, reactive arthropathy, infectious or post-infectious arthritis, gonococcal arthritis, tuberculous arthritis, viral arthritis, fungal arthritis, syphilitic
5 arthritis, Lyme disease, arthritis associated with "vasculitic syndromes", polyarteritis nodosa, hypersensitivity vasculitis, Luegenec's granulomatosis, polymyalgia rheumatica, joint cell arteritis, calcium crystal deposition arthropathy, pseudo gout, non-articular
10 rheumatism, bursitis, tenosynovitis, epicondylitis (tennis elbow), carpal tunnel syndrome, repetitive use injury, neuropathic joint disease (Charcot's joint), hemarthrosis (hemarthrosic), Henoch-Schönlein Purpura, hypertrophic osteoarthropathy, multicentric reticulohistiocytosis,
15 surcoilosis, hemochromatosis, sickle cell disease and other hemoglobinopathies, hyperlipoproteinemia, hypogammaglobulinemia, hyperparathyroidism, acromegaly, familial Mediterranean fever, Behçet's Disease, systemic lupus erythematosus, hemophilia, relapsing polychondritis,
20 and cystic fibrosis. The method comprises administering to a human having such diseases a therapeutically effective amount of the compound formula (I).

The compound of the invention, ((3-(2-amino-1,2-dioxoethyl)-1-((1,1'-biphenyl)-3-ylmethyl)-2-methyl-1H-indol-4-yl)oxy)acetic acid morpholino-N-ethyl ester is
25 believed to be a prodrug that transforms in the body of a mammal into ((3-(2-amino-1,2-dioxoethyl)-1-((1,1'-biphenyl)-3-ylmethyl)-2-methyl-1H-indol-4-yl)oxy)acetic acid. This acid is a known sPLA₂ inhibitor as shown by the following
30 data (from U.S. Patent No. 5,654,326):

Assay 1

A chromogenic assay procedure was used to evaluate ((3-(2-amino-1,2-dioxoethyl)-1-((1,1'-biphenyl)-3-ylmethyl)-2-

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methyl-1H-indol-4-yl)oxy)acetic acid as an inhibitor of recombinant human secreted phospholipase A₂. A general description of this assay method is found in the article, "Analysis of Human Synovial Fluid Phospholipase A₂ on Short Chain Phosphatidylcholine-Mixed Micelles: Development of a Spectrophotometric Assay Suitable for a Microtiterplate Reader", by Laure J. Reynolds, Lori L. Hughes, and Edward A. Dennis, Analytical Biochemistry, 204, pp. 190-197, 1992 (the disclosure of which is incorporated herein by reference):

10

Table I	
Compound of formula (II)	Inhibition of human secreted PLA ₂ IC ₅₀ ± mean deviation
	(3-4 tests) 4.33 ± 2.31 nM

Conclusion: The compound of formula (II) is highly active in inhibiting sPLA₂.

15

Assay 2
A guinea pig lung tissue procedure was used evaluate ((3-(2-amino-1,2-dioxoethyl)-1-((1,1'-biphenyl)-3-ylmethyl)-2-methyl-1H-indol-4-yl)oxy)acetic acid (compound of formula II) as an inhibitor of recombinant human secreted phospholipase A₂.

20

To estimate the drug-induced suppression of the maximal responses, sPLA₂ responses (10 ug/ml) were determined in the absence and presence of drug, and percent suppression was calculated for each pair of tissues.

25

Results of Human Secreted Phospholipase A₂ Inhibition Tests on guinea pig lung tissue

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Table II

Compound of formula (II)	Tissue test secreted PLA ₂ Apparent K _B nM
	57 ± 11

Assay III

The bioavailability of the compound of the invention,
5 ((3-(2-Amino-1,2-dioxoethyl)-1-((1,1'-biphenyl)-3-ylmethyl)-
2-methyl-1H-indol-4-yl)oxy)acetic acid morpholino-N-ethyl
ester, was determined using a Rat Study Protocol Plasma
Pharmacokinetics Study:

The purpose of this assay was to evaluate and compare the
10 oral delivery for selected sPLA₂ inhibitors.

Test Subject:

Species: Rat

Strain: Fischer 344

Dose Preparation:

15 The amount of sPLA₂ inhibitor was corrected for free acid
equivalents.

Vehicle:

Suspension of sPLA₂ inhibitor in 10% Acacia, prepared just
prior to dose administration

20

Dose Administration:

Route: Oral

Frequency: Single dose

Dose: 10 mg/kg (of the parent acid)

25 Dosage Volume: 5 mL/kg

Rats fasted overnight.

Specimen Collection:

Blood samples (0.8 ml) were obtained at the following times:

30 0.5, 1, 2, 4, 8 and 24 hours (2 rats/timepoint)

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Data Analysis:

Plasma was assayed by HPLC to measure concentrations of the different sPLA₂ inhibitors (measured as free acids).

- 5 C_{max} (maximal plasma concentrations), and AUC values were determined from the mean plasma concentration-time profiles.

Table 3

Compound ester type	C _{max} (ng/ml)	AUC (0-8hr)
morpholino-N-ethyl ¹	1163	5192
methyl ²	201	1129
ethyl ³	56	241
pivalate ⁴	98	361
isopropyl ⁵	491	2570
N,N- diethylglycolamido ⁶	751	3398

- 10 ¹ = ((3-(2-amino-1,2-dioxoethyl)-1-((1,1'-
biphenyl)-3-ylmethyl)-2-methyl-1H-indol-4-yl)oxy)acetic acid
morpholino-N-ethyl ester (compound of the invention)

- ² = ((3-(2-amino-1,2-dioxoethyl)-1-((1,1'-
biphenyl)-3-ylmethyl)-2-methyl-1H-indol-4-yl)oxy)acetic acid
15 methyl ester (this ester was evaluated in a separate study)

³ = ((3-(2-amino-1,2-dioxoethyl)-1-((1,1'-
biphenyl)-3-ylmethyl)-2-methyl-1H-indol-4-yl)oxy)acetic acid
ethyl ester

- ⁴ = ((3-(2-amino-1,2-dioxoethyl)-1-((1,1'-
20 biphenyl)-3-ylmethyl)-2-methyl-1H-indol-4-yl)oxy)acetic acid
pivalate ester

⁵ = ((3-(2-amino-1,2-dioxoethyl)-1-((1,1'-
biphenyl)-3-ylmethyl)-2-methyl-1H-indol-4-yl)oxy)acetic acid
isopropyl ester

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⁶ = ((3-(2-amino-1,2-dioxoethyl)-1-((1,1'-biphenyl)-3-ylmethyl)-2-methyl-1H-indol-4-yl)oxy)acetic acid N,N-diethylglycolamido ester

- 5 Table 3 shows that the morpholino-N-ethyl ester, the compound of the invention is unexpectedly more bioavailable than other esters of the sPLA₂ inhibitor, ((3-(2-amino-1,2-dioxoethyl)-1-((1,1'-biphenyl)-3-ylmethyl)-2-methyl-1H-indol-4-yl)oxy)acetic acid.
- 10 While the present invention has been illustrated above by certain specific embodiments, it is not intended that these specific examples should limit the scope of the invention as described in the appended claims.

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WE CLAIM:

1. The compound, ((3-(2-amino-1,2-dioxoethyl)-1-((1,1'-biphenyl)-3-ylmethyl)-2-methyl-1H-indol-4-yl)oxy)acetic acid morpholino-N-ethyl ester.

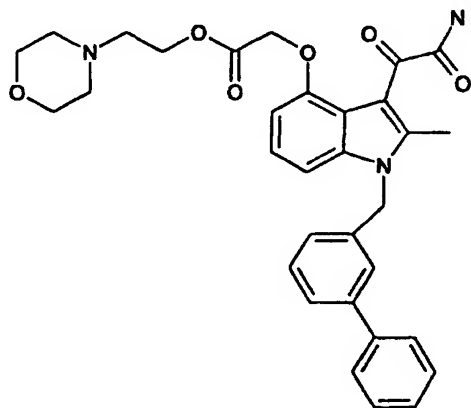
5

2. A pharmaceutical formulation comprising the compound of claim 1 together with a pharmaceutically acceptable carrier or diluent therefor.

10 3. A method of inhibiting sPLA₂ mediated release of fatty acid which comprises contacting sPLA₂ with an therapeutically effective amount of the compound as claimed in claim 1.

15 4. The method of claim 3 wherein the contacting sPLA₂ is done by oral administration of the compound as claimed in claim 1.

20 5. Use of the 1H-indole-3-glyoxylamide compound represented by formula (I);



(I)

for the manufacture of a medicant for therapeutic treatment of septic shock, adult respiratory distress syndrome,
25 pancreatitis, trauma, bronchial asthma, allergic rhinitis,

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reactive arthropathy, infectious or post-infectious arthritis, gonococcal arthritis, tuberculous arthritis, viral arthritis, fungal arthritis, syphilitic arthritis, Lyme disease, arthritis associated with "vasculitic syndromes", polyarteritis nodosa, hypersensitivity vasculitis, Luegenec's granulomatosis, polymyalgin rheumatica, joint cell arteritis, calcium crystal deposition arthropathris, pseudo gout, non-articular rheumatism, bursitis, tenosynovitis, epicondylitis (tennis elbow), carpal tunnel syndrome, repetitive use injury, neuropathic joint disease (charco and joint), hemarthrosis (hemarthrosic), Henoch-Schonlein Purpura, hypertrophic osteoarthropathy, multicentric reticulohistiocytosis, surcoilosis, hemochromatosis, sickle cell disease and other hemoglobinopathries, hyperlipo proteineimia, hypogammaglobulinemia, hyperparathyroidism, acromegaly, familial Mediterranean fever, Behat's Disease, systemic lupus erythrematosis, hemophilia, relapsing polychondritis, and cystic fibrosis; wherein the method comprises administration to said mammal a therapeutically effective amount of the compound as claimed in claim 1.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US98/22679

A. CLASSIFICATION OF SUBJECT MATTER

IPC(6) :A61K 31/535; C07D 413/12

US CL :544/144; 514/235.2

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 544/144; 514/235.2

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
CAS ONLINE

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X,P	Chemical Abstracts, volume 129, December 1998 (Columbus, OH, USA), the abstract no. 298393, MACIAS, W. I., "Method for treatment of chronic bronchitis." WO 9842343 A1, 01 October 1998.	1-4, 6

☐ Further documents are listed in the continuation of Box C.☐ See patent family annex.

* Special categories of cited documents:

A document defining the general state of the art which is not considered to be of particular relevance

E earlier document published on or after the international filing date

L document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

O document referring to an oral disclosure, use, exhibition or other means

T document published prior to the international filing date but later than the priority date claimed

T later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

X document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

Y document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

A document member of the same patent family

Date of the actual completion of the international search

06 JANUARY 1999

Date of mailing of the international search report

20 JAN 1999

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Form PCT/ISA/210 (second sheet)(July 1992)*

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US98/22679**Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)**

This international report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:
2. ☒ Claims Nos.: 5
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

The "Use of" format in the claim is not in compliance with PCT Article 6.
3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest.
☐ No protest accompanied the payment of additional search fees.

Form PCT/ISA/210 (continuation of first sheet)(1)(July 1992)*